SEROLOGIC DIAGNOSIS OF SCHISTOSOMIASIS

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IN 1961 Kagan and Pellegrino[1] published a comprehensive review of immunological methods for the diagnosis of schistosomiasis. In the succeeding years the study of serologic methods has continued. The relative sensitivity of serologic methods in infected persons living in Puerto Rico was studied by Anderson and Naimark,[2] who used four intradermal antigens and five serologic techniques and found, in contrast to most other observers, that the intradermal test was less sensitive in the detection of antibody. The complement-fixation (CF) test was 96 to 97 per cent sensitive with adult and cercarial antigens, respectively; the cercarial slide flocculation test was 98 per cent positive; the cercarial agglutination test was 98 per cent positive; and the circumoval precipitin test was 88 per cent positive for 355 infected subjects.

Kagan *et al.*[3] examined 103 apparently healthy asymptomatic Puerto Rican federal prisoners in Atlanta and found 29 per cent positive for eggs of *S. mansoni* by stool examination and rectal biopsy. Of these 103 individuals, only 45 were skin-test positive. Serologic study indicated the CF test to be the most sensitive serologic method (94 per cent) in detecting antibodies in the group passing eggs; the circumoval precipitin test gave an 89 per cent positive rate, hemagglutination 85 per cent, and cercarial slide flocculation a 77 per cent rate.

In 1960 the fluorescent antibody test was introduced for the diagnosis of schistosomiasis.[4] In 1964 Buck and his co-workers,[5] in an extensive survey in Ethiopia, evaluated intradermal CF, slide flocculation, the charcoal slide test, and the fluorescent antibody (FA) procedure. All serologic tests except for the FA procedure correlated well with parasitologic findings of schistosome eggs in the stool. The CF test again was found to be the most sensitive technique.

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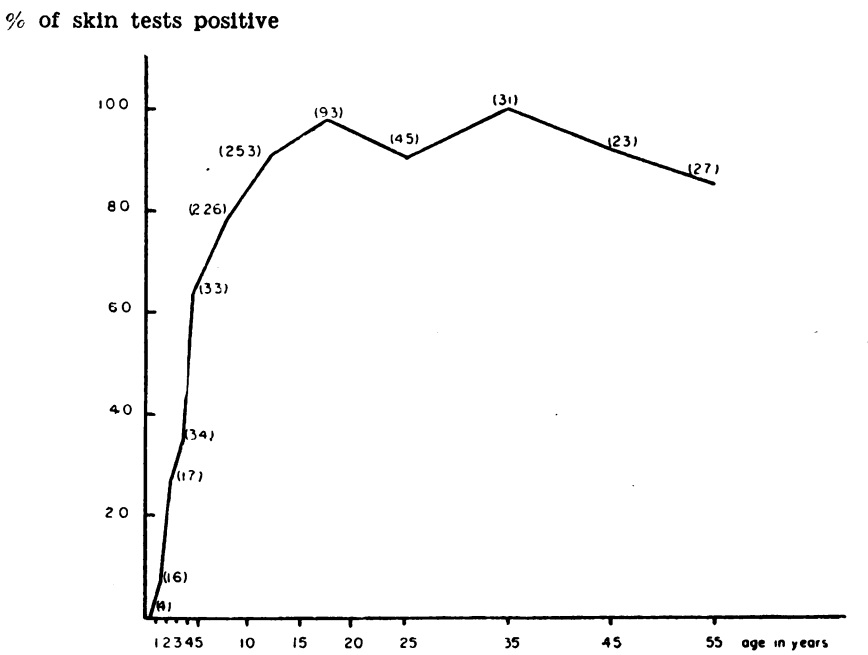

Fig. 1. Mean wheal areas of skin reactions to *S. mansoni* antigen in a population sample of Aduwa, Ethiopia. Reproduced by permission from Buck *et al.* Comparative studies of some immunologic screening tests for schistosomiasis in Ethiopia. *Ethiopian Med. J. 3:* 93-106, 1965.

The Intradermal Test

The intradermal test (IDT) for schistosomiasis, in my opinion, has reached the stage of being an evaluated diagnostic technique. Reproducible results can be obtained with diagnostic reagents prepared in different laboratories[6] when the antigens have been standardized with regard to nitrogen content and when the recommendations of the World Health Organization for performance of the procedure are followed. Extensive use by the Peace Corps in Africa in screening volunteers suggests that for maximum specificity rigid adherence to details of technique must be followed. The distribution of the areas of control wheals is a good barometer of the technique employed. If control wheal areas vary considerably from a mean area of 0.4 to 0.6 cm.^{2}, faulty technique is suspected and many false positive tests will be obtained.

Studies carried out with skin test antigens prepared in my laboratory have been made in many parts of the world. In Aduwa, Ethiopia,[7]

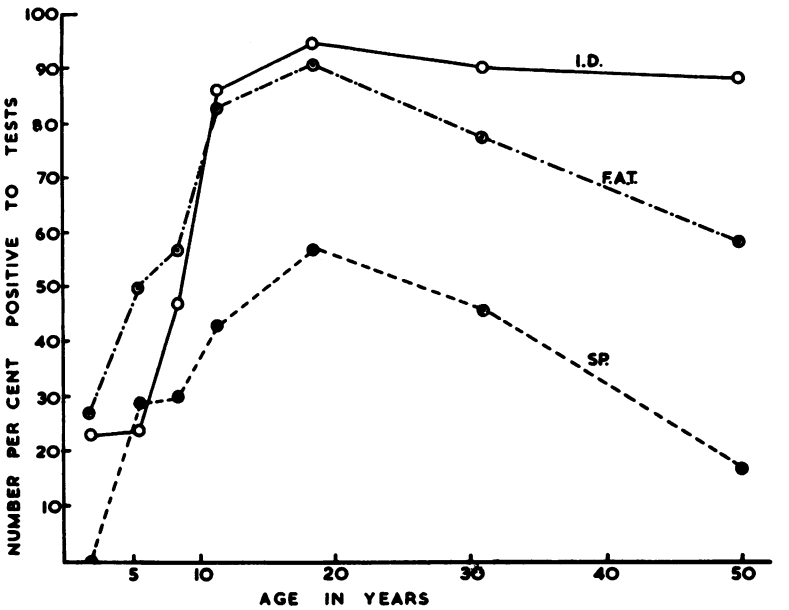

Fig. 2. Comparison of age-prevalence of *S. haematobium* and *S. mansoni* infections assessed by specimen examinations (SP.) with results of intradermal tests (I.D.) and fluorescent antibody tests (F.A.T.) in the community of the R.A.N. Mine, Rhodesia. Reproduced by permission from Clarke, V. de V., The prevalence of bilharzia in Rhodesia, *S. Afr. J. Med. Sci. 62*:229-36, 1966.

the results indicate 100 per cent skin sensitivity by the age of 20 (Figure 1). In Rhodesia[8] a similar pattern was obtained (Figure 2). In studies carried out in Nigeria,[9] the IDT was 93 per cent sensitive in patients passing eggs of *Schistosoma haematobium.* The test was found sensitive in recent studies in the Sudan. In one endemic area 100 per cent skin sensitivity was revealed.[10] In a group of 142 infected Bantu children on a citrus estate, 96 per cent (137 of 142) were positive by the skin test.[11] All the children were passing eggs of *S. haematobium* or *S. mansoni.*

The skin test can also be used as an epidemiologic tool. A recent study in Puerto Rico confirmed every known endemic area and pinpointed two areas that had not been recognized as hyperendemic for many years[12] (Figures 3 and 4).

The recent study of Kloetzel and Rodriguez da Silva[13] on the development of skin reactivity is especially important for an understanding of the test. Skin reactivity develops slowly in individuals living in endemic areas. The poor skin reactivity of young persons is a re-

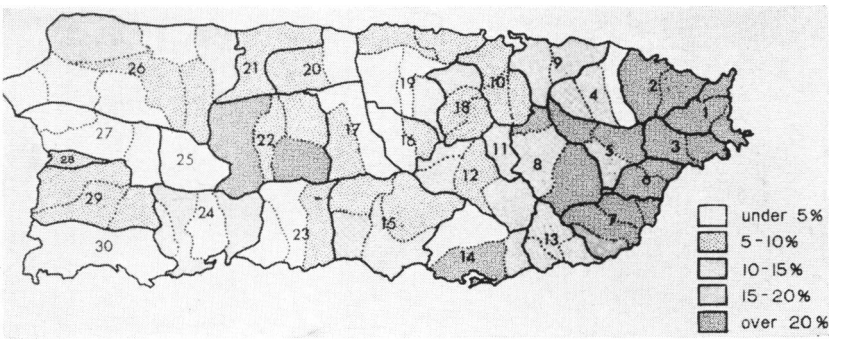

Fig. 3. Prevalence of schistosomiasis in rural subwatershed areas in Puerto Rico. Reproduced by permission from Kagan *et al. A Skin Test Survey for the Prevalence of Schistosomiasis in Puerto Rico*, Public Health Service Publication No. 1525. Washington, D.C., Govt. Print. Off., 1966.

flection of the time lived in the endemic area rather than of immunological incompetency. Adult males who have resided in an endemic area in Brazil for varying periods of time show the same skin reactivity as individuals born in an endemic area. The skin reactivity in Brazil for persons of all ages is not too different from the reactivity noted in Africa.

In a recent study carried out in Cadillac, Mich.[14] on 103 persons with histories of schistosome dermatitis, 24 to 28 per cent were reactive to the skin test with adult and cercarial antigens. Of the sera from these individuals, 7 per cent were positive in the cercarial slide flocculation test, 22 per cent in the FA test, and none in the CF test. This study confirms previous reports in the literature that exposure to nonhuman schistosome species will lead to reactivity in the intradermal test. The skin test is, therefore, a measure of recent and past experience with schistosome cercariae. A positive test indicates that where visceral schistosomiasis is under consideration, parasitologic studies to demonstrate eggs of the parasite should be undertaken. Treatment should not be initiated on the basis of a positive skin test.

The Fluorescent Antibody Test

Of the recently developed serologic tests for schistosomiasis, the FA test is a promising procedure. The evaluations of the technique by Sadun and Anderson[4, 15] and the use of plasma extracted from filter paper[16, 17] make the technique a useful diagnostic and epidemiologic tool. The test, however, is highly technical and requires careful standard-

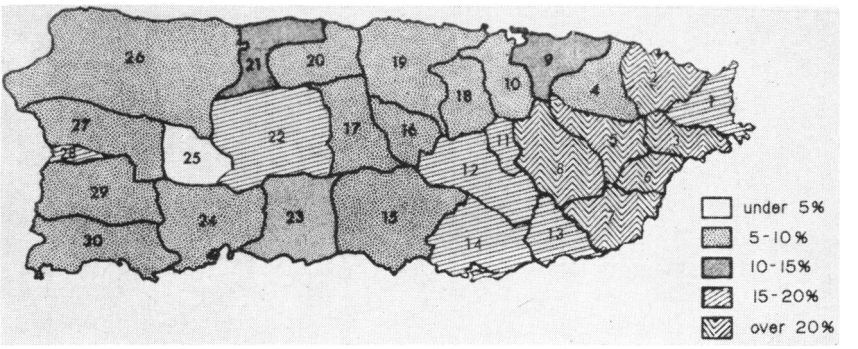

Fig. 4. Combined urban and rural prevalence rates for schistosomiasis in Puerto Rico. Reproduced by permission from Kagan *et al., A Skin Test Survey for the Prevalence of Schistosomiasis in Puerto Rico,* Public Health Service Publication No. 1525. Washington, D.C., Govt. Print. Off., 1966.

ization. In our laboratory we have found that the technique can be made very sensitive or insensitive by adjusting the concentration of the rhodamine bovine albumin in the preparation of the cercarial antigen. Care must also be used in evaluating the fluorescein conjugate employed, because concentration of this reagent affects the sensitivity of the test. In our evaluation of the technique[18] we found the test to be slightly less sensitive than early reports (75 to 80 per cent) and to give what we interpreted as "false positive" tests (32 to 40 per cent) in a group of Puerto Ricans who were negative by other parasitologic and serologic tests. Pellegrino[19] found the test to be 86 per cent sensitive in 307 patients with active schistosomiasis compared to 96 per cent in the CF and 92 per cent in the flocculation test.

In the recent study on sera from persons who had schistosome dermatitis,[14] the FA test, with a cercarial antigen, was positive in 22 per cent. The test, therefore, has to be used with care, since exposure to avian schistosomes will incite production of detectable antibodies. This may explain the so-called "nonspecific" FA results obtained in Ethiopia[5] and our positive results in "negative" individuals in Puerto Rico.[18] We also obtained a high rate of nonspecific positive tests with a group of sera from Tahiti—an area where schistosomiasis is not endemic but where avian and marine schistosomes do exist.

Another pitfall in the technique is the presence of minute amounts of the patient's gamma globulin when the test is carried out in tubes. After three to four washings of cercariae in buffer, traces of free gamma globulin from a positive serum will quench the reaction

markedly.[20] This is the basis for a prozone phenomenon reported by some workers.

A final source of error is the sensitivity of the technique employed. With sera from Peace Corps volunteers, a sensitive test gave too many false positive reactions. The sensitivity was adjusted and a less sensitive technique gave closer correlation with other serologic procedures employed in our laboratory. The sensitive and insensitive FA techniques were employed on a battery of 200 sera from Somalia. These sera were obtained from an endemic area where single urine examinations indicated a prevalence of bilharziasis of approximately 40 per cent. The cercarial slide flocculation test gave a prevalence rate of 31 per cent, a bentonite flocculation test gave a prevalence rate of 56 per cent, the sensitive FA test 56 per cent, and the insensitive test 17 per cent. In this instance the sensitive test gave a close approximation of the true prevalence of the infection. These are some of the problems that must be resolved in the use of the FA technique. In Africa the use of FA tests for diagnosis is creating a problem because a positive test is being used as the basis for treatment. Before the test can be used to greatest advantage the above mentioned variables must be standardized.

The Cercarial Slide Flocculation Test

The cercarial slide flocculation (CL) test employing cholesterol-lecithin crystals coated with lipid-free cercarial antigen was described by Anderson.[21] Evaluation of this technique[2, 22] and the use of extracts of schistosome eggs and water-soluble secretions and excretions[23] have extended its usefulness. The technique in our hands has proved to be both reliable and useful. There are, however, some problems. In a laboratory that receives sera through the mail from all parts of the world, contaminated or chylous sera are sometimes received. The particulate matter in such sera is difficult to distinguish from the antigen-antibody reaction with the cholesterol particle antigen. A certain percentage of the sera cannot be evaluated by this technique because, in control tests with centrifuged serum without the addition of antigen, "false" agglutination, or what looks like agglutination, takes place.

Another problem is the reactivity of the test with sera from patients with other parasitic infections. In a recent evaluation of 44 sera from children in Cherokee, N. C., 10, or 23 per cent, were reactive in the test. There is no schistosomiasis in North Carolina, but some of these

TABLE I—THE SENSITIVITY AND SPECIFICITY OF THE BENTONITE FLOCCULATION TEST FOR SCHISTOSOMIASIS*

Human sera tested *Diagnosis*	*No. reactive* *No. tested*	*Per cent reactive*
Schistosomiasis	16/20	80
Trichinosis	15/24	63
Visceral larva migrans	7/18	39
Syphilis	1/8	13
Echinococcosis	2/19	11
Filariasis	1/4	7
Swimmer's itch	2/95	2
Toxoplasmosis	0/7	0
Amebiasis	0/3	0
Cysticercosis	0/1	0
Kala-azar	0/3	0
Viral infections	0/9	0
Normal	1/57	2

*From Chisholm *et al.*[25]

TABLE II—RESULTS OF SEROLOGIC TESTS WITH HUMAN SERA FROM SCHISTOSOMIASIS INFECTIONS IN RHODESIA*

Tests	*No. tested*	*Per cent reactive*
Flocculation tests:		
Bentonite	90	57
Cholesterol-lecithin slide	93	68
Charcoal card	93	55
Complement-fixation test	84	48
Fluorescent-antibody test	93	57
Indirect hemagglutination test	93	47

*From Chisholm *et al.*[25]

children were infected with *Ascaris*. The test also cross-reacted strongly with sera from individuals who had trichina infections. In many parts of the world this is no problem since trichinosis is not endemic, but it still is a problem in the United States and in Puerto Ricans living in New York City.

Pellegrino[24] evaluated the technique on 3,688 sera. In this group the sensitivity of the test was 90 per cent in parasitologically confirmed cases and 79 per cent in children living in a hyperendemic area. The specificity ranged from 94 to 98 per cent. In our hands the CL test has

not been as sensitive or specific. With the sera from asymptomatic Puerto Rican prisoners the test was 77 per cent sensitive.[3] With 93 sera from Rhodesia, from individuals passing eggs of *S. mansoni* or *S. haematobium*, 68 per cent were reactive. With sera of 119 people in Michigan the test gave 7 per cent nonspecific results. With sera from children heavily laden with intestinal parasites a nonspecific reactive rate of 23 per cent was obtained.[25] The test is still the one used in the routine examination of more than 1,000 sera received each year in our laboratory for the diagnosis of schistosomiasis.

Bentonite Flocculation Test for Schistosomiasis

To overcome some of the difficulties in reading the cercarial slide flocculation test, the same antigen was absorbed onto bentonite particles. The reactivity of this bentonite flocculation test (BFT) with selected sera was compared with results obtained in the cholesterol lecithin slide flocculation, the indirect hemagglutination test (IHA), charcoal slide, CF, and FA tests.[25] The results obtained in evaluating a battery of sera for sensitivity and specificity are shown in Table I. Note the high reactivity with the schistosomiasis sera and the low reactivity with the sera from individuals exposed to nonhuman schistosome cercariae. A strong cross-reaction with trichina was expected. The relatively high level of reactivity with visceral larva migrans (VLM) sera was also observed. Evaluation of a group of sera from Rhodesia (Table II) shows that the bentonite test compares favorably with the FA and the charcoal card test but is not as sensitive as the cercarial slide flocculation test. In spite of the shortcomings in the test, we feel it is a useful addition to the diagnostic armamentarium since it expands the usefulness of the flocculation test.

Charcoal Plasma Card Test

The adaptation of the plasma card test for schistosomiasis[26] made it possible to employ the flocculation reaction under field conditions. I have evaluated the Hynson, Westcott, and Dunning kit for schistosomiasis in Africa. The results in this instance were unsatisfactory because of the age of the antigen. With fresh antigen the sensitivity and specificity are excellent. With old antigen the charcoal tends to clump spontaneously, making the test hard to read. Care must be taken to use dated antigen.

Complement-Fixation Test

With the proper antigen the complement-fixation (CF) test is probably the best serologic procedure for the diagnosis of schistosomiasis. There have been many efforts to improve the antigen from Chaffee's[27] early effort.[28, 29, 30, 31, 32] In our laboratory we have successfully adapted the CF test to the microtitration method[33] with good results. This microtitration method makes it possible to perform hundreds of tests with very small amounts of serum.

The CF test in our hands has not been as sensitive as other procedures. The reasons for this have not been assessed. We have adapted the Laboratory Branch Complement-Fixation (LBCF) test[33] for the routine diagnosis of many parasitic infections, and the loss of sensitivity may be due to the technique of the test, the amount of complement used (5-C'_{50} units), or the microtitration method. Other workers have not had this problem. Dunston and Pepler[34] in South Africa were able to employ *S. matheei* as a source of antigen and reported 93 per cent sensitivity. Rifaat and Khalil[35] prepared antigens from *Fasciola gigantica*, *Schistosoma bovis*, and *S. mansoni*. Best results were obtained with delipidized schistosome antigens, the *S. mansoni* extracts being more sensitive than the *S. bovis* antigen. The sensitivity of a good CF test for schistosomiasis should be between 85 and 95 per cent for cases proved positive.

Pautrizel *et al.*[36] evaluated a conglutinin complement absorption test for schistosomiasis and found the technique both sensitive and specific. Recently Antunes and Pellegrino[37] reevaluated the technique utilizing an adult worm antigen and found the test 100 per cent specific and 94 per cent sensitive.

The Circumoval Precipitin Test

A circumoval precipitin (COP) test was described by Oliver-González in 1954.[38] By 1960 there were at least 16 papers published on it by many different authors. To date active interest in this test has been maintained. Bruijning[39] in Holland carried out extensive work on experimental schistosomiasis in hamsters. He was able to detect COP antibodies in very lightly infected animals (one pair of worms). Fraga de Azevedo and Rombert[40] in Portugal obtained a sensitivity of 80 to 90 per cent with sera of individuals infected with *S. mansoni* and *S. haematobium* in Mozambique, Portuguese Guinea, and Brazil. There were,

however, a few false positive reactions observed if one considered a + reaction as positive. Guy-Grand *et al.*[41] recommend the technique in France, and the outstanding work of Cancio and Rodriguez-Molina in Puerto Rico[42] has done much to provide insight into the mechanism of the reaction. The use of lyophilized eggs[43] makes this test practical for the first time since the quality and the source of the eggs are very important in influencing the sensitivity of the technique. The test has one serious drawback since it will not detect antibodies in unisexual infections. Reyes and Yogore[44] found the COP test very useful in diagnosing cerebral schistosomiasis japonicum in the Philippines. They utilized cerebrospinal fluid to incubate the eggs. Patients with intestinal schistosomes gave positive COP reactions with serum but negative reactions with spinal fluid. The test with spinal fluid is very specific for involvement of the central nervous system.

The Cercarienhullen Reaction

The cercarienhullen reaction (CHR) test is an *in vitro* procedure that utilizes living cercariae. Jordan and Goatly[45] found the reaction useful in detecting antibody in the younger group (5- to 7-year-old children were 86 per cent positive) whereas the infected older persons (40 to 49 years of age) were 43 per cent positive. Guy-Grand *et al.*[41] found in an acquired infection that the CHR became positive before the COP reaction and the appearance of eggs in the stools. Fraga de Azevedo and Rombert[40] obtained in the test a sensitivity of 50 to 96 per cent with the highest reactivity in young groups. In their hands the specificity of the test was very high. Since the CHR reaction must be made with living, infectious cercariae, the test is not too useful as a routine procedure.

Indirect Hemagglutination Test

The indirect hemagglutination (IHA) test for schistosomiasis was initially evaluated by me[46] in Oliver-González' laboratory in Puerto Rico.[47] We used this technique for diagnosis for many years in the United States but stopped performing it because the sensitivity with sera from missionaries returning to this country was not equal to that of other tests. With sera from individuals living in endemic areas such as Puerto Rico, Africa, and Brazil, results of the test were within expected sensitivity.

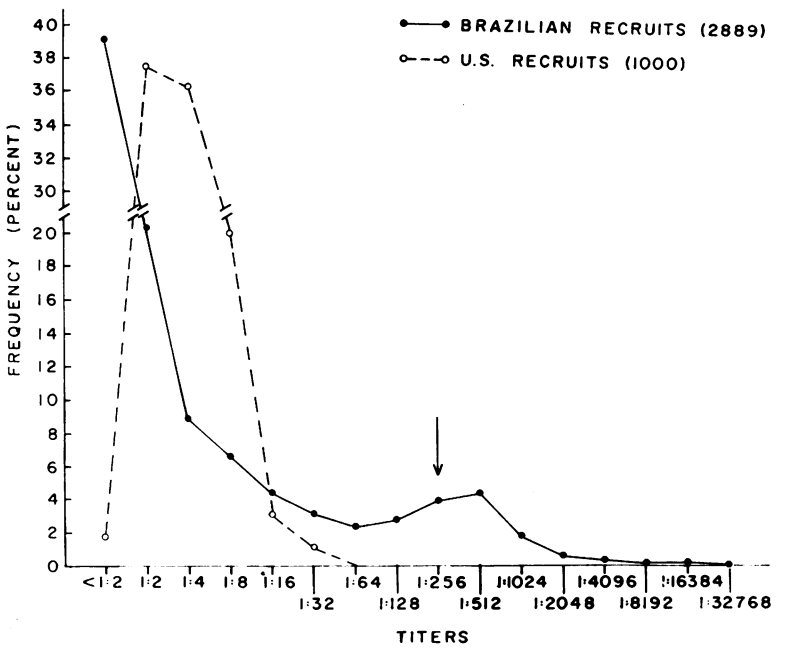

Fig. 5. Frequency of hemagglutinating titers to *S. mansoni* in United States and Brazilian military recruits. Reproduced by permission from Cuadrado, R. R. and Kagan, I. G. The prevalence of antibodies to parasitic diseases with sera of young army recruits from the United States and Brazil. *Amer. J. Epidem. 86*:330-40, 1967.

We have returned to the use of the IHA in our seroepidemiological studies.[48] In Figure 5 note the frequency of titers from 3,000 U.S. military recruits and 3,000 Brazilian recruits. The geographic distribution of the positive Brazilian reactors agrees very nicely with the known distribution of schistosomiasis in Brazil with one or two exceptions. We are in the process of evaluating this test with groups of sera from Colombia and Argentina. There is, to my knowledge, no schistosomiasis in Colombia or Argentina and it will be of interest to ascertain the type of curves obtained with collections of sera obtained in these countries.

T'ien-Wei *et al.*[49] developed a slide indirect hemagglutination test with formalin-preserved sheep erythrocytes sensitized with an extract of eggs of *S. japonicum*. In evaluating 185 proved cases of schistosomiasis the test was found to be 96 per cent sensitive, and with 522 healthy nonschistosomiasis individuals the test was 3 per cent nonspecific. The sensitivity of the test was higher than that of the CHR reaction.

Discussion

The immunologic tests available for the diagnosis of schistosomiasis will detect antibody in the serum of 70 to 90 per cent of infected individuals, depending on their backgrounds and clinical histories. The specificity is within acceptable limits. There are areas, however, that require further study. The antigens employed are complex. Purification is limited to partial delipidization in most instances. Antigenic analysis of delipidized extracts of cercariae, adult worms, and metabolic secretions and excretions indicates a multitude of antigenic components. Further research is needed.

Research is needed to determine the type of antibody being measured in our tests. Preliminary work in our laboratory on the fractionation of immune serum into IgM, IgG, and IgA components has shown that the 7s-IgG and the IgA components are active in the cercarial slide flocculation test. The IgG fraction is active in the CHR reaction and in the passive cutaneous anaphylaxis (PCA) test in the guinea pig; the IgA complex is active in the PCA test in the monkey. 2-Mercaptoethanol will inactivate the FA reaction, which suggests that perhaps IgM is active; however, we have been unable to demonstrate this directly.

A third area of interest is the reactivity of serologic tests for epidemiologic studies of populations living in an endemic area. Serologic evaluations have been carried out with hospital and clinic populations to evaluate tests for diagnostic purposes. These studies carry a certain amount of bias by virtue of the fact that they deal with a particular part of the population.

What is the effect of the immune state on serologic tests? Can one detect antibody as readily in an immune population as in a hospital population? Recent studies in our laboratory suggest that the sensitivity of serologic tests is lower in such an endemic population. Schofield[50] studied the sensitivity of the CF test in populations with residence in Africa of less than 3 years, of 3 to 10 years, and of more than 10 years. He reported that the sensitivity of the test was 100 per cent in the less-than-3-year group, 71 per cent in the 3-to-10 year group, and 44.7 per cent in the 10 plus year group.

What is the sensitivity of the test in a transient population? In a group of several thousand furloughed missionaries tested in our laboratory, only 31 of 53 (60 per cent) passing eggs of *S. mansoni* or *S. haematobium* were positive.[51]

Active work on reagin antibodies is in progress in several laboratories.[52, 53, 54, 55] In this regard, studies on PK (Prausnitz-Küstner) antibodies indicate that this type of antibody in the serum is more readily demonstrated than the precipitin type. Mendes and Amato[56] tested the sera of 15 patients with schistosomiasis in Brazil and found the PK tests more reliable. Ivey[57] has shown that PCA tests are more sensitive for the diagnosis of ascariasis and *Toxocara* infections than routine serologic tests. Autoantibodies were present in 27 per cent of cases of schistosomiasis.[58]

The role of heterophile antibodies was investigated by Antunes and Pellegrino.[59] These antibodies were found to have no practical value in the diagnosis of schistosomiasis since their presence could be detected with schistosome antigen in the serum of most nonschistosome patients. Further work on the role of nonprecipitating cell-fixed antibody is needed. We have an active research program in progress in our laboratory at the present time in this area.

There is a need to develop serologic methods for the evaluation of the effect of chemotherapy. Studies on monkeys[60] and man[61] indicate that reliable serologic methods to assess chemotherapeutic cure are not available. Dodin *et al.*[62] observed a detectable circulating antigen in the serum of patients on the seventh day of treatment. Assessment or circulating antigen by a reverse serologic procedure (coating a particle carrier with specific antibody) or by gel diffusion methods may prove useful.

In conclusion, much remains to be accomplished in the serology of schistosomiasis in spite of the excellent work reported in the literature.

Summary

The serologic and immunologic diagnosis of schistosomiasis is carried out by the following procedures: intradermal tests (IDT), fluorescent antibody (FA), cercarial slide flocculation (CL), bentonite flocculation (BFT), charcoal plasma card test, complement fixation (CF), circumoval precipitin (COP), indirect hemagglutination (IHA), and cercarienhullen reaction (CHR). The very recent literature is reviewed and the value of the tests as they are carried out for diagnostic and epidemiologic purposes in my laboratory are discussed. Areas requiring further research are outlined. Data obtained in the evaluation of a new BFT for schistosomiasis are presented.

REFERENCES

1. Kagan, I. G. and Pellegrino, J. A critical review of immunological methods for the diagnosis of bilharziasis. *Bull. W.H.O. 25*:611-74, 1961.
2. Anderson, R. I. and Naimark, D. H. Serologic diagnosis of *Schistosoma mansoni* infections. II. Sensitivity of intradermal and serologic tests on individuals with an unequivocal diagnosis of schistosomiasis. *Amer. J. Trop. Med. 9:* 600-03, 1960.
3. Kagan, I. G., Rairigh, D. W. and Kaiser, R. L. A clinical, parasitologic and immunologic study of schistosomiasis in 103 Puerto Rican males residing in the United States. *Ann. Intern. Med. 56:* 457-70, 1962.
4. Sadun, E. H., Williams, J. S. and Anderson, R. I. Fluorescent antibody technic for serodiagnosis of schistosomiasis in humans. *Proc. Soc. Exp. Biol. Med. 105*:289-91, 1960.
5. Buck, A. A., Sadun, E. H., Anderson, R. I. and Shaffa, E. Comparative studies of some immunologic screening tests for schistosomiasis in Ethiopia. *Amer. J. Hyg. 80*:75-84, 1964.
6. Kagan, I. G., Pellegrino, J. and Memoria, J. M. P. Studies on the standardization of the intradermal test for the diagnosis of bilharziasis. *Amer. J. Trop. Med. 10*:200-07, 1961.
7. Buck, A. A., Spruyt, D. J., Wade, M. K. and Deresse, A. and Feyssa, E. Schistosomiasis in Adwa. A report on an epidemiological pilot study. *Ethiopian Med. J. 3*:93-106, 1965.
8. Clarke, V. de V. The prevalence of bilharzia in Rhodesia. *S. Afr. J. Med. Sci. 62*:229-36, 1966.
9. Pi-Sunyer, F., Gilles, H. M. and Wilson, A. M. M. *Schistosoma haematobium* infection in Nigeria. 1. Bacteriological and immunological findings in the presence of schistosomal infection. *Ann. Trop. Med. Parasit. 59*:304-11, 1965.
10. Khalil, H. M. Prevalence of schistosomiasis in Gazira, Sudan, as revealed by the intradermal test. *Trans. Roy. Soc. Trop. Med. Hyg. 61*:100-03, 1967.
11. Kagan, I. G., Kaiser, R. L. and Kent, N. Comparison of *Schistosoma mansoni, S. japonicum,* and *Trichinella spiralis* antigens in skin tests on persons with schistosomiasis mansoni and haematobium. *Amer. J. Trop. Med. 15*:719-24, 1966a.
12. Kagan, I. G., Negron, H., Arnold, J. C. and Ferguson, F. F. *A Skin Test Survey for the Prevalence of Schistosomiasis in Puerto Rico.* Public Health Service Publication No. 1525, Washington, D. C., Govt. Print. Off., 1966b, 91 pp.
13. Kloetzel, K. and Rodriguez da Silva, J. Schistosomiasis mansoni acquired in adulthood: Behavior of egg counts and the intradermal test. *Amer. J. Trop. Med. 16*:167-69, 1967.
14. Moore, G. T., Kaiser, R. L., Lawrence, R. S., Putnam, S. M. and Kagan, I. G. Intradermal and serologic reactions to antigens from *Schistosoma mansoni* in schistosome dermatitis. In press.
15. Anderson, R. I., Sadun, E. H. and Williams, J. S. Preserved cercariae in the fluorescent antibody (FA) test for schistosomiasis. *Exp. Parasit. 11*:226-30, 1961.
16. Anderson, R. I., Sadun, E. H. and Williams, J. S. A technique for the use of minute amounts of dried blood in the fluorescent antibody test for schistosomiasis. *Exp. Parasit. 11*:111-16, 1961.
17. Sadun, E. H., Anderson, R. I. and Williams, J. S. Fluorescent antibody test for the laboratory diagnosis of schistosomiasis in humans by using dried blood smears on filter paper. *Exp. Parasit. 11*:117-20, 1961.
18. Kagan, I. G., Sulzer, A. J. and Carver, K. An evaluation of the fluorescent antibody test for the diagnosis of schistosomiasis. *Amer. J. Epidem. 81:* 63-70, 1965.
19. Pellegrino, J. Diagnostico sorológico da Esquistossomose mansónica. 1. Estudo comparativo entre as reações de floculação, de immunofluorescência e de fixação do complemento. *Rev. Inst. Med. Trop. S. Paulo 5*:147-53, 1963.
20. Sulzer, A. J. and Kagan, I. G. Indirect

fluorescent antibody (IFA) tests for parasitic diseases. III. Conjugate-antigen relationships in the tests for trichinosis and schistosomiasis. *Amer. J. Med. Techn. 33*:1-8, 1967.

21. Anderson, R. I. Serologic diagnosis of *Schistosoma mansoni*. 1. Development of a cercarial antigen slide flocculation test. *Amer. J. Trop. Med 9*:299-303, 1960.
22. Jachowski, L. A. and Anderson, R. I. Evaluation of some laboratory procedures in diagnosing infections with *Schistosoma mansoni*. *Bull. W.H.O. 25*: 675-93, 1961.
23. Sadun, E. H., Schoenbechler, M. J. and Bentz, M. Multiple antibody response in *Schistosoma mansoni* infections: Antigenic constituents in eggs, cercariae, and adults (excretions and secretions) determined by flocculation reactions, cross absorption and double diffusion studies. *Amer. J. Trop. Med. 14*:977-95, 1964.
24. Pellegrino, J. Diagnóstico sorológico da Esquistossomose mansónica. IV. Valor da reação de floculação em levantamentos epidemiologicos. *Rev. Inst. Med. Trop. S. Paulo 6*:111-13, 1964.
25. Chisholm, E. S., Allain, D. S. and Kagan, I. G. The standardization and evaluation of the bentonite flocculation test for the diagnosis of schistosomiasis. In preparation.
26. Sadun, E. H., Anderson, R. I. and Schoenbechler, M. J. A plasma card test for rapid serodiagnosis of schistosomiasis (SPC). *Proc. Soc. Exp. Biol. Med. 112*:280-83, 1963.
27. Chaffee, E. F., Bauman, P. M. and Shapilo, J. J. Diagnosis of schistosomiasis by complement fixation.
28. Schneider, M. D. and Radke, M. G. Further observations on a complement fixing susbtance from *Schistosoma mansoni*. *Proc. Helminthol. Soc. Wash. 24*: 137-39, 1957.
29. Schneider, M. D., Radke, M. G. and Coleman, M. T. Immunologically reactive substance from *Schistosoma mansoni*. *Exp. Parasit. 4*:391-97, 1956.
30. Sadun, E. H., Lin, S. S. and Walton, B. C. Studies on the host parasite relationships to *Schistosoma japonicum*. III. The use of purified antigens in the diagnosis of infections in humans and experimental animals. *Milit. Med. 124*: 428-36, 1959.
31. Sleeman, H. K. Isolation and study of a specific complement fixing antigen from adult *Schistosoma mansoni*. *Amer. J. Trop. Med. 9*:11-17, 1960.
32. Sleeman, H. K., Burke, J. D. and Kent, J. F. Physicochemical and serologic studies of *Schistosoma mansoni* antigens. *Amer. J. Trop. Med. 7*:241, 1958.
33. Casey, H. Standardized diagnostic complement fixation method and adaptation to micro test. In: *Public Health Monograph No. 74*. Washington, D.C., Govt. Print. Office, 1965.
34. Dunston, T. H. J. and Pepler, W. J. A new complement fixation test for schistosomiasis. *S. African Med. J.* 162-64, Feb. 20, 1965.
35. Rifaat, M. A. and Khalil, H. M. The use of antigens prepared from adult *Fasciola gigantica, Schistosoma mansoni* and *Schistosoma bovis* in the diagnosis of urinary schistosomiasis by the complement fixation test (CFT). *J. Egypt. Public Health Ass. 40*:197-205, 1965.
36. Pautrizel, R., Tribouley, J., Szersnovicz, F. and Duret, J. Application de la réaction de conglutination au diagnostic des bilharzioses. *Ann. Inst. Pasteur 107*:863-72, 1964.
37. Antunes, L. J. and Pellegrino, J. Serological diagnosis of *Schistosoma mansoni* infection. *Bull. W.H.O. 35*:721-25, 1966.
38. Oliver-Gonzalez, J. Anti-egg precipitins in the serum of humans infected with *Schistosoma mansoni*. *J. Infect. Dis. 95*: 86-91, 1954.
39. Bruijning, C. F. A. The circumoval precipitate reaction in experimental schistosomiasis, II. *Trop. Geogr. Med. 16*:256-62, 1964.
40. Fraga de Azevedo, J. and Rombert, P. C. On the value of the cercariae membrane reaction (CHR) and the circumoval precipitin reaction (COP) in the diagnosis of bilharziasis. *Ann. Soc. Belg. Med. Trop. 44*:239-54, 1964.
41. Guy-Grand, B., Pasquier, P., Tutin, M.

and Baur, H. Bilharziose intestinale de première invasion. Diagnostic sérologique (à propos d'une observation). *Presse. Med. 44*:2471-74, 1965.

42. Cancio, M., de Sala, A. R., Rodriguez-Molina, R. and Toro-Goyco, E. *In vitro* quantitation of the circumoval preciptin test in infections with *Schistosoma mansoni*. *Exper. Parasit. 16*:64-73, 1965.
43. Sala, A. R., Cancio, M. and Rodriguez-Molina, R. Preservation of eggs of *Schistosoma mansoni* for the circumoval preciptin test, *Amer. J. Trop. Med. 11*: 199-200, 1962.
44. Reyes, V. A. and Yogore, M. G. On cerebral schistosomiasis with a new diagnostic test. *Philipp. J. Surg. 18*: 172-76, 1963.
45. Jordan, P. and Goatly, K. D. Observations on pericercarial reactions of sera in schistosomiasis in Tanganyika. *Trans. Roy. Soc. Trop. Med. Hyg. 57*:184:90, 1963.
46. Kagan, I. G. Hemagglutination after immunization with schistosome antigens. *Science 122(3165)*:376-77, 1955.
47. Kagan, I. G. and Oliver-Gonzalez, J. Hemagglutination studies with schistosome antigens, *J. Parasit. 44*:457-60, 1958.
48. Cuadrado, R. R. and Kagan, I. G. The prevalence of antibodies to parasitic diseases with sera of young army recruits from the United States and Brazil. *Amer. J. Epidem. 86*:330-40, 1967.
49. T'ien-wei, H., Teh-Fu, T., Chien-Liang, C., and Ch'eng-chou, H. Indirect hemagglutination test (slide method) in the diagnosis of schistosomiasis japonica. *Chin. Med. J. 85*:96-106, 1966.
50. Schofield, F. D. The schistosomal complement-fixation test. Part I. The diagnostic value. *Trans. Roy. Soc. Trop. Med. Hyg. 53*:64-74, 1959.
51. McQuay, R. M. Parasitologic studies in a group of furloughed missionaries. II. Helminth findings. *Amer. J. Trop. Med. 16*:161-66, 1967.
52. Ogilvie, B. M. Reagin-like antibodies in animals immune to helminth parasites. *Nature 204*:91-92, 1964.
53. Ogilvie, B. M., Smithers, S. R. and Terry, R. J. Reagin-like antibodies in experimental infections of *Schistosoma mansoni* and the passive transfer of resistance. *Nature 209(5029)*:1221-23, 1966.
54. Zvaifler, N. J., Sadun, E. H. and Becker, E. L. Anaphylactic (reaginic) antibodies in helminth infections, *Clin. Res. 14*:336, 1966.
55. Hsü, H. F. and Li Hsü, S. Y. Reagin-like antibody in rhesus monkeys immune to *Schistosoma japonicum*, *Z. Tropenmed. Parasit. 17*:166-76, 1966.
56. Mendes, E. and Amato Neto, V. Some immunological aspects regarding schistosomiasis mansoni: The passive antibody transference test and precipitin test. *Hospital (Rio) 64*:9-13, 1963.
57. Ivey, M. H. Immediate hypersensitivity and serological responses in guinea pigs infected with *Toxocara canis* or *Trichinella spiralis*. *Amer. J. Trop. Med. 14*: 1044-51, 1965.
58. Shamma, A. H., Ali, A. J. T. and El-Shawi, N. N. Auto-antibodies in *Schistosoma haematobium* infections. *J. Path. Bact. 90*:659-61, 1965.
59. Antunes, L. J. and Pellegrino, J. Heterophile antibodies in *Schistosomiasis mansoni*. *J. Parasit. 53*:303-05, 1967.
60. Jachowski, L. A., Anderson, R. I. and Sadun, E. H. Serologic reactions to *Schistosoma mansoni*. I. Quantitative studies on experimentally infected monkeys (*Macaca mulatta*). *Amer. J. Hyg. 77*:137:45, 1963.
61. Sadun, E. H., Anderson, R. I., DeWitt, W. B. and Jachowski, L. A. Serologic reactions to *Schistosoma mansoni*. II. Quantitative studies in human patients treated with Stibophen. *Amer. J. Hyg. 77*:146-49, 1963.
62. Dodin, A., Ratovondrahety, Moureau, J. P. and Richaud, J. Etude immunologique de bilharziens traités par le CIBA 32644-Ba. *Acta Tropica (Supp. 9)*:35-44, 1966.